PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mark C. Fishman et al.	Confirmation No.:	8749
Serial No.:	10/656,873	Art Unit:	1634
Filed:	September 5, 2003	Examiner:	Jehanne Souaya Sitton
Customer No.:	21559		
Title:	Methods for Diagnosing and Treating Heart Disease		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MARK C. FISHMAN, M.D., AND XIAOLEI XU, PH.D.
UNDER 37 C.F.R. § 1.131

We declare:

1. We are the inventors of the subject matter that is described and claimed in the above-captioned patent application.

2. The enclosed Exhibit is a copy of laboratory notebook pages, which show that we determined that the pickwick mutation, which is characterized by a weak heartbeat, is in the titin gene. In particular, we found that certain zebrafish sequences that we had identified as being in the pickwick locus were homologous to known titin sequences. These pages are dated prior to the August, 1999 publication date of Satoh et al. (Biochem. Biophys. Res. Com. 262:411-417, 1999). This work was carried out in the United States of America.

3. All statements made herein of our own knowledge are true, and all statements made on information and belief are believed to be true, and further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date:_____ _____

Mark C. Fishman, M.D.

Date:_____ _____

Xiaolei Xu, Ph.D.

pickwick | positional cloning

LG9 | 1803p marker use strong/well.

5cM
10789. (8 Re/1,000) → 1 YAC
Connexin 500 embryo → 3 YAC. by primers
7cM.
from 3'UTR. of connexin
as superpool.

[1] went on ~~to~~ use the primer pair to screen the 8 plate pool

Total 24 PCR reaction. 12 8 / 8. ~~30 x 3 = 90~~.

. Genomic DNA 1 675 50X. 3 g/λ use 3λ

order . cas → en lable. 35 cycle X90

25λ.	Template	4λ	(1 ng/λ)	2.6.
	10X A	2.5 λ		225
	25mM dNTP.	0.1 λ.		9
17.8 / 90 / 1602 → 6.	primer	0.25 λ.	20mM.	22.5
	primer	0.25		22.5
	Taq	0.1 λ		9
	H_2O 4:	17.8 λ.		1602
		25 λ.		

1. IVF. fish do not give/squeeze out eggs. try next week. select strong fish!
try with my m646 wt fish although AB/TL background. at least. get something.

2.

Titin

pickwick could be titin. zch256 show high homologe with titin (connectin). which makes sense.

1. Z8363 scan all mutants to identify recombinants. > 500 embryos. confirm with z20031 about 5m ID'd.

2. design primers from af 036118. do [redacted] PCR together with zch256 against Y5. Y6. hope to pick up the rigth side about Y5T3. Y6T3

3. Compare human/mouse/chick/ titin sequence. design primer pairs against the 27 kb cDNA (conserved region

① put into RH map. confirm its identity.

② PCR against Y5. Y6.

③ isolate EAC. & get the intron. 3'UTR. region and then design primers for SSCP.

[redacted]

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1. Got embryos for mV062H (their parents are het's!
   m686.9 x TL ♂ (two pairs)
   mV010H
   m521A (1 # are low)
   mP18a (TL allele).

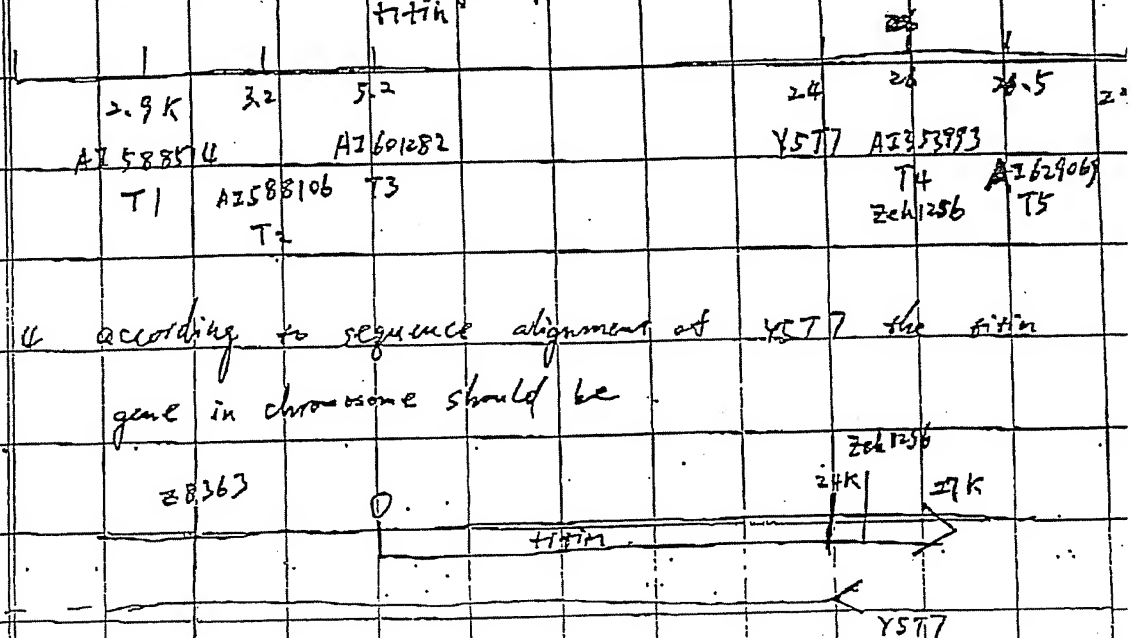
Today bleached five out of 6 except m521A

next Tuesday put them into system.

Tommorrow look at phenotype

More good news!

1. from EST project 4 were titin zebrafish version!
2. Y5T7 end [redacted] is titin homologue!
3. Best of all. they represents different portions of titin



4. according to sequence alignment of Y5T7 the titin gene in chromosome should be

WITNESS:

DATE: